PB86-216199

Test Battery for Screening Behavioral Teratogens in Mice

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Prepared for

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-85/420	2.
3. PB86-216199	
4. TITLE AND SUBTITLE A TEST BATTERY FOR SCREENING BEHAVIORAL TERATOGENS IN MICE (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Mary Jeanne Kallman[1] and Lyman W. Condie, Jr.[2]	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS [1]Departments of Psychology and Pharmacology, University of Mississippi, University, MS 38677 [2]Toxicology and Microbiology Division, HERL/ORD/USEPA, Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. ABMC1A
	11. CONTRACT/GRANT NO. In-house
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory (HERL) Office of Research and Development (ORD) U.S. Environmental Protection Agency (USEPA) Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Journal
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES P.O. Lyman Condie, TOTB/TMD/HERL/ORD/USEPA (CI)
Neurobehavioral Toxicology and Teratology, Vol. 7, No. 6, 727-731, Nov/Dec 1985

16. ABSTRACT

The assessment battery was developed for identifying teratogens in mice (CD-1, outbred strain) since many non-behavioral toxicologists prefer working with the mouse and because the mouse is less costly to house and feed. This approach emphasizes the importance of adult toxicity data in the selection of exposure levels for teratologic studies and for interpreting behavioral teratogen effects. Potential teratogen exposure is both prenatal and postnatal with the goal of identifying the need for more costly and time consuming, cross--fostering and critical period designs. The test battery includes the assessment of multiple behavioral capabilities, including physical landmarks, reflex development, motor capability and passive avoidance learning. We have found the behavioral capabilities of the mouse to be similar to those of the rat in variability and range of response. Our methodology has emphasized drinking water exposure to chemicals since we were involved in the evaluation of drinking water contaminants but the battery should be amenable to any route of exposure.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 7
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

EPA/600/J-85/420
JOURNAL ARTICLE

Neurobehavioral Toxicology and Teratology, Vol. 7, pp. 727–731, 1985. © Ankho International Inc. Printed in the U.S.A. 0275-1380/85 $3.00 + .00

A Test Battery For Screening Behavioral Teratogens in Mice[1,2]

MARY JEANNE KALLMAN[3]

Departments of Psychology and Pharmacology, University of Mississippi, University, MS

AND

LYMAN W. CONDIE, JR.

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH

KALLMAN, M. J. AND L. W. CONDIE, JR. *A test battery for screening behavioral teratogens in mice.* NEUROBEHAV TOXICOL TERATOL 7(6) 727–731, 1985.—The assessment battery was developed for identifying teratogens in mice (CD-1, outbred strain) since many non-behavioral toxicologists prefer working with the mouse and because the mouse is less costly to house and feed. This approach emphasizes the importance of adult toxicity data in the selection of exposure levels for teratologic studies and for interpreting behavioral teratogenic effects. Potential teratogen exposure is both prenatal and postnatal with the goal of identifying the need for more costly and time consuming, cross-fostering and critical period designs. The test battery includes the assessment of multiple behavioral capabilities, including physical landmarks, reflex development, motor capability and passive avoidance learning. We have found the behavioral capabilities of the mouse to be similar to those of the rat in variability and range of response. Our methodology has emphasized drinking water exposure to chemicals since we were involved in the evaluation of drinking water contaminants, but the battery should be amenable to any route of exposure.

Teratology Screening Behavior Behavioral teratology Drinking water Reflex development Motor ability Mice

OUR approach to behavioral teratologic screening was developed as part of a large interdisciplinary toxicology project to evaluate potential toxicity of drinking water contaminants for which there were little or no available data. This project was supported by the U.S. Environmental Protection Agency so that these data could be used in setting guidelines for maximum exposure levels for compounds which existed in drinking water [17]. Most of our efforts were devoted to assessing the potential for toxicity in the adult. Because the prenatal period has been identified by numerous investigators as a particularly sensitive period for producing toxicity [6, 11, 18, 21] we included a teratologic assessment procedure as part of our overall evaluation. Not only is the prenatal period a sensitive time for producing toxicity but this period may be the only time when toxicity occurs for some chemicals [11,22].

METHOD

Animal Model

The selection of an appropriate animal model was the first consideration which we confronted. The decision making was motivated by many factors other than solely preferences for a specific animal model for behavioral testing. For example, the immunologists vehemently supported using the mouse model since many of the basic evaluation procedures for assessing immunologic function were not well validated for the rat.

The mouse model posed both some advantages and some problems for us. Advantages included reduced cost for the mouse model as compared to the rat model. Acquisition and housing costs were roughly one-half of the same cost for the rat. Moreover, the adult mouse could be efficiently group-housed as opposed to single-housing which was required for the rat. The most serious disadvantage for using the mouse in behavioral testing was the absence of a broad behavioral data base for the mouse in the late 70's and the absence of standardized behavioral test equipment for mouse assessment. Fortunately, the feasibility of doing behavioral teratologic research was supported by data on prenatal development of the mouse [12], reproductive physiology [8,18], and mating behavior of the mouse [8]. Moreover, since the

[1]Supported in part by cooperative agreements 806481 and 80861010 from the U.S. Environmental Protection Agency, Health Effects Laboratory, Cincinnati, OH.

[2]This paper has not been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies, and therefore its contents do not necessarily reflect the view of the agency and no official endorsement should be inferred.

[3]Requests for reprints should be addressed to Dr. Mary Jeanne Kallman, Department of Psychology, University of Mississippi, University, MS 38677.

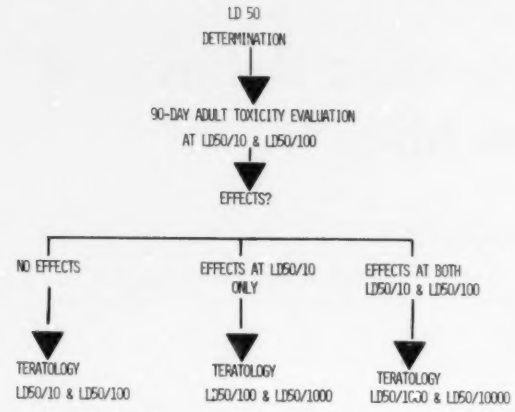

FIG. 1. The dosing flow chart used to make decisions about doses for initial behavioral teratology studies.

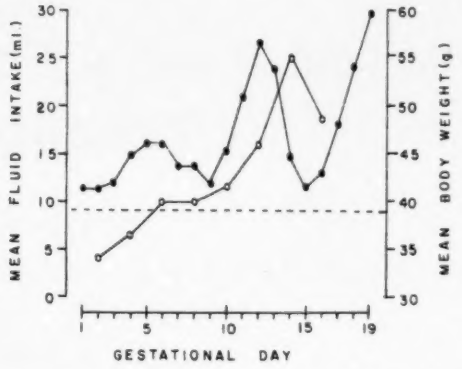

FIG. 2. Mean fluid intakes and mean body weights throughout the 19 day gestational period (N=8). Fluid intakes are represented by solid circles and were measured daily. Mice were weighed every other day during gestation and mean body weights are represented by the open circles. No weight data appear for day 18 because some mothers were in the process of delivering on day 18. The dashed line on the graph represents mean adult intakes (9 ml) and mean body weights (39 g) used to approximate exposure levels.

late 70's a number of laboratories has been involved in behavioral research with the mouse (e.g., [2, 3, 10, 13, 16, 25, 26]).

The data obtained for toxicity testing in adult mice was used to make decisions about dose selection in teratology studies which occurred at a later time point in our screening sequence. These data were also a tremendous asset in determining the feasibility of maternal drinking water exposure and in avoiding prenatal exposure at concentrations which would result in maternal weight reductions and overt changes in maternal behavior. In general, behavioral teratology investigations should be conducted at chemical exposure levels which produce no physical or behavioral changes for the mother and no physical abnormalities in the pups. Since behavioral evaluation can be costly and time consuming, it should be conducted at exposure levels which do not produce easily detected physical abnormalities.

Chemical Exposure

The method of chemical exposure for both our teratology and adult toxicity studies was intended to simulate drinking water exposure as much as possible. Therefore, we provided drinking water exposure whenever the contaminant of interest was soluble and relatively stable (less than 10% loss) across 3–4 days. Besides being most relevant to human population exposures, drinking water exposure reduced the stress and trauma associated with daily gavage as well as the technical support required for contaminant delivery to pregnant mice.

Previous work indicated that relatively simple taste tests could be used to predict whether a contaminant could be placed in drinking water for chronic 24 hr availability and not reduce the total fluid intake of adult mice [14,15]. These data indicated that unless a solution was seriously avoided in a two-bottle, choice situation where both tap water and the contaminant were available (contaminant intake=20% or less of total fluid consumed) that fluid intake would be normal in the forced drinking situation. Data obtained from the adult screening were used to estimate concentrations for drinking solutions to provide maternal exposures for the teratology investigations. It was suspected that fluid intakes for pregnant dams would be different from adult intakes, but no data were available to describe actual fluid intakes on a daily basis during the gestational period. Chemical solutions were prepared on the basis of an average adult body weight of 39 g and an average daily fluid intake of 9 ml per day.

Dosing Regimen

The dosing regimen was designed to maximize the likelihood of identifying a problem if it existed with the intention of later determining the critical exposure period which produced the change. Therefore contaminant exposures were to both the male and to the female for 30 days prior to mating, during mating and to the female throughout gestation, and until the pups were weaned at 21 days of age. From the time of birth until 21 days of age when weaning occurred, the pups had access to the contaminated solution via nursing and by direct access to the drinking solutions. Since pups will begin to supplement nourishment provided by nursing with available food and water prior to weaning, access to the drinking solutions was possible.

Initial screenings were accomplished by exposure to three contaminant levels: a non-treated control, a group exposed to approximately one-tenth of the known adult acute LD50 and a group exposed to approximately one-hundredth of the LD50 when these exposure levels did not produce behavioral toxicity in the adult upon chronic exposures. If toxicity had been observed for the adult at these levels then teratology studies were conducted at lower doses. The flow chart in Fig. 1 illustrates the process used to make teratology dosing decisions. The LD50 was selected as the most important factor for determining exposure levels since in the absence of any teratologic or adult toxicity, guidelines would be established on the basis of lethality data [17].

TABLE 1

HISTORICAL DATA FOR CONTROL PREGNANCIES IN MICE*

Observation	Normal Values Mean ± S.E.M.
Mean No. pups/litter	13.2 ± 1.0
Mean pup weight (g)	1.53 ± 0.06
Gestational length	18.7 ± 0.18
Gestational wt. gain (g)	20.6 ± 2.48
	Frequency
Stillborn pups	1
Gross malformations	0
Pup deaths (days 1–21)	3

*Data based on 8 litters.

Historical Data

A number of variables were examined prior to the time of behavioral assessment of the pups to substantiate physical deficits of the mothers or gross physical abnormalities of the pups at the time of delivery. Maternal weights, food intake and fluid intakes were examined throughout the investigation. When the litter was delivered, the following information was obtained: number of pups in the litter, pup weights, gestational length, gestational weight gain of the mother, number of stillborn pups and malformed pups. All historical data collected on the mothers and pups were obtained to help clarify whether physical changes might account for observed behavioral changes when they occurred. All litters were culled to 8 pups at this time.

Behavioral Testing

Due to the limited behavioral repertoire of the neonatal mouse, we selected behavioral tests which had all been used previously to evaluate young rodents. Reflex development has been used frequently for the evaluation of both neonatal rats and mice [12, 20, 23, 24, 27]. We selected those reflexes and physical "landmarks" which could be quantified successfully to determine intensity and which could be observed reliably. Most investigators had previously used a nominal scale for observing the presence or absence of landmark development [12]. An alternative 6-point rating scale was developed by Burkhalter and Balster [5] which included many of the previously used behavioral capabilities which could be quantified. Burkhalter and Balster [5] describe in detail the methodology for quantifying these responses. In general, all of these observations are assigned rank values dependent on the duration or intensity of pup response on each of eight different measures.

The reflex development battery included assessment of eye opening, righting, forelimb placing, forepaw grasping, rooting, cliff drop, bar holding and auditory startle. Testing was conducted on neonatal pups from days 1 through 15 following birth. If any pup failed to respond (score=0) on any test, that pup was retested at the end of the day's testing. Rodent mothers, including mice, will cannabalize young pups in response to unique olfactory cues [12] emitted by the pups. Thus, for the purpose of conducting behavioral testing, the mother was removed from the cage first and the experimenter wore surgical gloves when handling each pup for testing. The pups were kept warm by placing the cages under a light during the testing process and then the mother was returned to the cage when testing was completed. This handling procedure was successful since maternal infanticide was rarely observed. Because of staff limitations and the necessity of timing births within one week of each other, it was impossible to test all pups on a given day. The procedure employed was to randomly select three pups from each litter for testing on each postnatal day. Under other circumstances, identifying and testing each pup daily would provide the ideal data collection procedure.

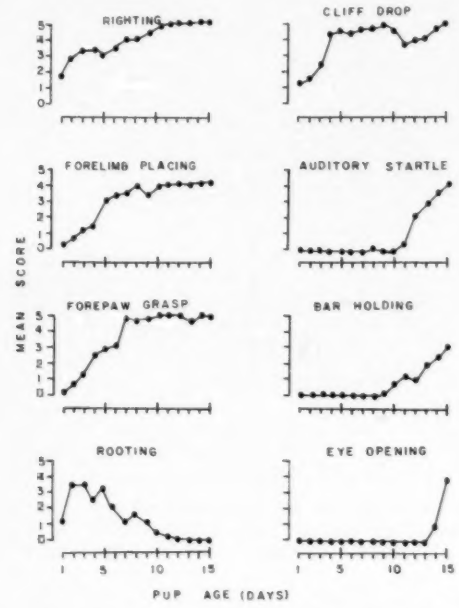

FIG. 3. The mean rating score for normal pups (N=15) on each of the different reflex measures across the 15 days following birth of the pups. Individual scores for each task was determined by observation of mouse performance and recording the quality of performance on a rating scale of 0–5. A score of "0" represented the absence of performance and a score of "5" represented excellent performance.

On day 17 the pups were assessed for muscular coordination with a procedure first described by Coughenour *et al.* [7]. Briefly, this test is conducted by placing a mouse on a wire-mesh pedestal (13×13 cm), inverting the pedestal 180°, thereby placing the mouse in an upside-down hanging position and measuring the length of time required for the mouse to climb to the top side of the screen pedestal.

On day 21 all pups were weaned from their mothers and a final behavioral assessment was conducted on days 23–24. Passive avoidance learning was assessed in a two-compartment chamber. The mouse was placed in the translucent side of the chamber and allowed to run into the dark compartment, foot shock (40 vAC) was delivered to the floor of the chamber. One hr and 24 hr after the training trial, mice were retested in the absence of shock delivery to determine latencies for entering the shock compartment. A maximum time of 180 sec could elapse before the trial was terminated if no response was made.

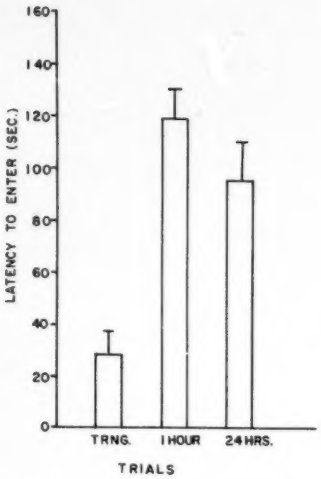

FIG. 4. Mean latencies (+ S.E.M.) to enter the passive avoidance shock compartment on the training trial and on the 1 hr and 24 hr test trials. Data presented in the graph were obtained from control mice (N=15).

Data Analysis

All of the data collected in the behavioral teratology evaluations were analyzed statistically. Maternal body weights, fluid intakes and food intakes were analyzed by analysis of variance procedures to examine the effects of treatment condition (vehicle, low dose and high dose of exposure) across days for each of the dependent measures. Significant main effects or significant interactions were further examined with multiple comparison procedures to identify specific differences. The rating data collected on the eight neurobehavioral reflexes or landmarks were analyzed by Friedman's two-way analysis of variance procedure for ranked interval data [19] to determine the effects of contaminant exposure across days of testing. Righting latencies on the screen test and latencies to enter the shock compartment on passive avoidance trials were log transformed ($\log_{10}$), as suggested by Edwards [9] to normalize time-based data, and analyzed by analysis of variance procedures. In all situations, a p-value≤ 0.05 was considered significant for concluding that a behavior was altered by chemical exposure.

RESULTS

Figure 2 depicts the fluctuations in fluid consumption and body weights by non-treated controls in a recent study conducted by us on the teratologic effects of oral exposure to chloral [15]. As can be seen in the figure, these data indicate that maternal fluid intakes are less stable than intakes for non-pregnant mice. Body weights and fluid intakes increased during gestation in the mouse and these increases are most dramatic between gestational days 10–15. Increases in fluid intake usually precede body weight gains and asymptote before body weights. Possibly a better approach to maintaining a consistent chemical exposure level throughout the gestational period would require adjusting the concentration of contaminated solutions at about day 10. Since fluid intakes increase dramatically at this time, the concentration would be decreased to maintain a constant exposure level.

Historical data collected from vehicle treated mice are typified by those presented in Table 1. Litter sizes, frequency of stillborn pups, and spontaneous rates of malformations observed for the mouse are quite comparable to those that have been observed in the rat.

Figure 3 illustrates the normal developmental sequence for each of the neurobehavioral reflexes for vehicle treated mice. As can be seen in the figure, each capability has a unique developmental sequence such that some responses become stronger or more sophisticated with maturation (i.e., grasping and auditory startle) while other capabilities decrease in intensity with maturation (i.e., rooting).

Data from vehicle treated mice indicates that normal 17 day old mice can successfully complete the screen test. Their mean latencies for righting are 34.4±5.5 sec. Neuromuscular coordination at this age is qualitatively similar to that which we have seen for adult mice on this same test procedure [15].

Figure 4 presents data collected from vehicle treated pups on the passive avoidance learning task. On the initial training trial, mice ran into the darkened compartment in about 28 seconds. After the delivery of shock on the training trial, subsequent test trials at 1 hr and 24 hr produced significantly increased latencies to enter the darkened compartment where shock was previously delivered.

DISCUSSION

The choice of the mouse as a model for behavioral teratology studies has been useful. When involved in toxicology research which is not limited to the potential for toxicity during the prenatal and early postnatal period, the mouse has many advantages. The major advantages include cost of the investigation and the ability to coordinate findings with other types of toxicology data where the mouse is typically used. The use of the mouse in behavioral research has suggested that the patterns and types of responses observed are similar to those which have been more extensively investigated in the rat [1, 13, 14, 15, 23].

The battery described employed oral chemical exposure because of the intended application of the data. The battery could be adapted for use with other routes or methods of exposure depending upon the interest of the investigators. Previous work in our laboratory indicates that the feasibility of drinking water exposure can be determined before executing an investigation [14,15] and that drinking fluid concentrations can roughly simulate an average daily level of exposure for repetitive dosing studies [14]. Other investigators could produce daily exposures more precise than those which we have obtained by making daily adjustments in fluid concentrations on the basis of weights and fluid intakes.

The selection of contaminant levels for exposure is at best difficult. Although behavioral teratology screening has not always been done with chemicals which are complete unknowns, designing investigations with unknown chemicals can necessitate numerous pilot studies to obtain estimates of relevant dose ranges for screening. The rationale described for dose selection is based on the premise that if adult lethality and toxicity data are not available then these data should be obtained prior to executing and interpreting a behavioral teratology study. From a regulatory perspective, the comparison of teratogenicity data to adult toxicity data would seem most logical.

The exposures to both the mothers and to the fathers

prior to mating and to the mother until the pups are weaned does not permit delineation of probable sources for toxicity that might have been observed. This was not the goal for the described battery but rather the intention was to use these investigations to identify chemicals which warranted further laboratory efforts. More recently, we have ceased paternal exposures because the spermatogenesis cycle is not inclusive with the 30 day premating exposure period [8]. We have been reluctant to justify the extra cost of housing and exposing animals for 60 days prior to mating.

The behavioral measures selected for inclusion in the battery are relatively simple to assess in neonatal mice. Clearly, other behavioral assessments might have been chosen to measure learning or motor performance or different behavioral capabilities might have been included. Probably the most difficult situations occur when attempting to conduct numerous behavioral tests or attempting to assess capabilities which are not well developed in young mice. Whenever possible, our screening batteries for both neonates and adults have included tests that might be considered composites, i.e., a test which requires multiple capabilities to produce success on the test. The reason for using these composite tests was that screening could be accomplished efficiently and deficits could be evaluated later to determine precise losses.

The battery described is an approach to evaluating unknown chemicals for the potential to produce behavioral teratogenicity in the mouse. This approach has been used successfully to screen halogenated hydrocarbons found in drinking water supplies [15], though it might be modified on many specific details depending on experimenter interest.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Drs. Robert L. Balster and Joseph F. Borzelleca for their encouragement throughout this project and for valuable discussion of these data.

REFERENCES

1. Balster, R. L. The effects of phencyclidine and three analogues on motor performance in mice. *Pharmacology* **20:** 46–51, 1980.
2. Balster, R. L. and B. Baird. Effects of phencyclidine, d-amphetamine and pentobarbital on spaced responding in mice. *Pharmacol Biochem Behav* **11:** 617–623, 1979.
3. Balster, R. L., V. C. Moser and W. L. Woolverton. Concurrent measurement of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J Pharmacol Methods* **8:** 299–309, 1982.
4. Bignami, G. Behavioral pharmacology and toxicology. *Annu Rev Pharmacol* **16:** 329–366, 1976.
5. Burkhalter, J. E. and R. L. Balster. Behavioral teratology evaluation of trichloromethane in mice. *Neurobehav Toxicol* **1:** 199–205, 1979.
6. Butcher, R. E., K. Howver, J. Burbacher and W. Scott. Behavioral effects from antenatal exposure to teratogens. In: *Aberrant Development in Infancy: Human and Animal Studies*, edited by N. R. Ellis. Hillsdale, NJ: Erlbaum, 1975, pp. 161–167.
7. Coughenour, L. L., J. R. McClean and R. B. Parker. A new device for rapid measurement of motor function. *Pharmacol Biochem Behav* **6:** 351–353, 1977.
8. Crispens, C. G. *Handbook on The Laboratory Mouse*. Springfield: Charles C. Thomas Publisher, 1975.
9. Edwards, A. *Experimental Design in Psychological Research*. New York: Rinehart, 1960.
10. Evans, H. L., A. M. Dempster and C. A. Snyder. Behavioral changes in mice following benzene inhalation. *Neurobehav Toxical Teratol* **3:** 481–485, 1981.
11. Fein, G. G., P. M. Schwartz, S. W. Jacobson and J. L. Jacobson. Environmental toxins and behavioral development. *Am Psychol* **38:** 1188–1197, 1983.
12. Fox, M. W. Reflex-ontogeny and behavioural development of the mouse. *Anim Behav* **13:** 234–241, 1965.
13. Glowa, J. R. Some effects of sub-acute exposure to toluene on schedule-controlled behavior. *Neurobehav Toxicol Teratol* **3:** 463–465, 1981.
14. Kallman, M. J. and G. L. Kaempf. Efficacy of choice testing to predict chronic ingestion of drinking solutions adulterated with chemicals. *Pharmacol Biochem Behav* **20:** 195–200, 1984.
15. Kallman, M. J., G. L. Kaempf and R. L. Balster. Behavioral toxicity of chloral in mice: An approach to evaluation. *Neurobehav Toxicol Teratol* **6:** 137–146, 1984.
16. Moser, V. C. and R. L. Balster. The effects of acute and repeated toluene exposure on operant behavior in mice. *Neurobehav Toxicol Teratol* **3:** 471–475, 1981.
17. National Academy of Sciences. *Drinking Water and Health*. Washington, DC: National Academy of Sciences, 1977.
18. Rodier, P. M. Postnatal functional evaluations. In: *Handbook of Teratology*, edited by J. G. Wilson and F. C. Fraser. New York: Plenum Press, 1976.
19. Siegal, S. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company, Inc., 1956.
20. Smart, J. L. and J. Dobbing. Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behavior in the rat. *Brain Res* **28:** 85–95, 1971.
21. Spyker, J. M. Behavioral teratology and toxicology. In: *Behavioral Toxicology*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1975, pp. 311–349.
22. Spyker, J. M. Assessing the impact of low level chemicals on development: Behavioral and latent effects. In: *Behavioral Pharmacology: The Current Status*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1976, pp. 161–180.
23. Van Abeelen, J. H. F. and J. T. R. Kalkhoven. Behavioural ontogeny of the nijmegen waltzer, a neurological mutant in the mouse. *Anim Behav* **18:** 711–718, 1970.
24. Wahlsten, D. A developmental time scale for postnatal changes in brain and behavior of B6D2F2 mice. *Brain Res* **72:** 251–264, 1974.
25. Webb, D. and T. C. Levine. Effects of caffeine on drl performance in the mouse. *Pharmacol Biochem Behav* **9:** 7–10, 1978.
26. Wenger, G. R. and P. Dews. The effects of phencyclidine, ketamine, d-amphetamine and pentobarbital on schedule controlled behavior in the mouse. *J Pharmacol Exp Ther* **196:** 616–624, 1976.
27. Wood, R. Reinforcing properties of inhaled substances. *Neurobehav Toxicol* **1:** Suppl 1, 67–72, 1979.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.